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BACTERIAL DESORPTION BY COMMERCIAL MOUTHWASHES *vs* TWO-PHASE OIL:WATER FORMULATIONS

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During the past eight years, various investigators have shown that oral microorganisms tend to have hydrophobic surface properties. Most oral microorganisms isolated directly from the oral cavity, as well as many oral laboratory strains, adhere to oil droplets. The authors have recently shown that certain concentrations of the cationic antibacterial agents, i.e. cetylpyridinium chloride (CPC) and chlorhexidine (CHX) promote binding of oral and other microorganisms to oil droplets. This has resulted in development of novel aqueous:oil two-phase mouthwashes containing CPC and/or CHX. In the present manuscript, two such formulations were compared with other commercial mouthwashes for their ability to desorb microorganisms from a solid surface (polystyrene). Of the various mouthrinses tested, only the oil:water formulations efficiently removed the adherent bacterial layer. The data suggest that oil:water mouthwashes may have desorption properties superior to those of many commercial mouthwashes.

KEY WORDS: Adhesion, desorption, mouthwash, polystyrene, oil

INTRODUCTION

Bacterial adhesion to oral surfaces is considered a prerequisite for subsequent colonization (Gibbons, 1984). Development of biofilms on soft (mucosal) oral surfaces is kept in check by continual desquamation of epithelial cells, together with their attached microbiota. However, bacterial accumulation on teeth quickly reaches macroscopic proportions (i.e. dental plaque).

One conceivable approach to limiting bacterial film formation in the oral cavity is to inhibit the initial adhesion process. Indeed, addition of certain sugars, surfactants and chaotropic agents can block adhesion of oral microorganisms to model surfaces, and to one another (McIntire *et al.*, 1982; Nesbitt *et al.*, 1982; E Rosenberg *et al.*, 1983). However, such agents are relatively ineffective in removing bacteria which are already attached. Methods of removing bound microorganisms thus appear to have greater potential. Whereas many studies have been dedicated to investigation of the *in vitro* antimicrobial effects of mouthwashes (Perdok *et al.*, 1989), few, if any, reports exist regarding their bacterial desorption properties.

In 1983, desorption of *Acinetobacter calcoaceticus* RAG-1 cells from polystyrene was reported, using simple oil:saline combinations (Rosenberg *et al.*, 1983). The authors have recently presented evidence that addition of certain cationic antibacterial agents (cetylpyridinium chloride [CPC] and chlorhexidine [CHX]) can greatly increase

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adhesion of microorganisms and oral debris to oil droplets (Goldberg *et al.*, 1990). This led to the premise that oil:water mouthrinse formulations containing such agents could serve as effective desorbing mouthrinses, with additional antimicrobial properties.

In the present manuscript, the *in vitro* bacterial desorption ability of two such oil: water formulations is compared with that of commercially available mouthrinses, using a modified *in vitro* technique (Rosenberg *et al.*, 1983).

MATERIALS AND METHODS

Acinetobacter calcoaceticus RAG-1 (ATCC 31012) cells were grown in brain heart infusion broth (Difco Ltd) to stationary phase (18 h) with shaking. Cells were harvested, washed twice and suspended in phosphate buffer (22·2 g $K_2HP0_4 \cdot 3H_2O$, 7·26 g KH_2PO_4 and distilled water to 1 l, pH 7·1).

One ml of a very turbid suspension of RAG-1 cells (O.D. = 9·3, 400 nm) was added to disposable polystyrene cuvettes, 1 cm square (4 ml capacity). Following vortexing for 1 min, the RAG-1 cells were poured off, leaving a film of adherent cells. The cuvettes were washed briefly with tap water. To each cuvette, 1 ml of mouthwash or water (control) was added (Fig. 1) and the cuvettes vortexed for 2 min. The mouthwash was poured off, 1 ml fresh mouthwash was added, and the cuvettes again vortexed for 2 min. The mouthwash was poured off, and the remaining bacterial film stained by adding gentian violet to the cuvettes for 10 min, followed by washing with water to remove excess stain. The remaining bacterial film was quantified by measuring the optical absorbance (585 nm) from both sides of the dried cuvettes in a Uvikon 710 spectrophotometer (Kontron, Zürich, Switzerland).

Results are presented as the average percent decrease in optical absorbance as compared to control cuvettes rinsed with water alone. The types of commercial mouthrinses used in the study, and their origin are presented in Table 1.

Odex is a propriety formulation provided by Agis Ltd consisting of an aqueous solution containing CPC, sweetener, and food colour, and an oil phase (15% v/v) containing a mixture of vegetable and essential oils. Odex+ contains, in addition, 0·025% chlorhexidine gluconate.

RESULTS

In the technique employed, polystyrene cuvettes are first coated with an adherent layer of *Acinetobacter calcoaceticus* RAG-1 cells. Adhesion of this microorganism to surfaces has been extensively studied (Rosenberg & Kjelleberg, 1986), and its avid adhesion to polystyrene has previously been demonstrated (Rosenberg, 1981; Rosenberg *et al.*, 1983). In the original technique, following desorption by oil:water mixtures, outgrowth of RAG-1 cells was used to quantify the residual layer of adherent microorganisms (Rosenberg *et al.*, 1983). Since the present formulations contain antibacterial agents, this approach was modified by using a staining procedure to measure residual adherent cells. This, in turn, entailed employing a much more turbid cell suspension in order to obtain a thick film of adherent microorganisms. Quantitation of desorption could then be ascertained spectrophotometrically.

Results of a typical experiment are summarized in Figure 2 and Table 2. Among the 12 mouthwashes tested, only Odex and Odex+ were highly effective in removing the dense bacterial film, desorbing 98% and 92%, respectively, as compared to the control

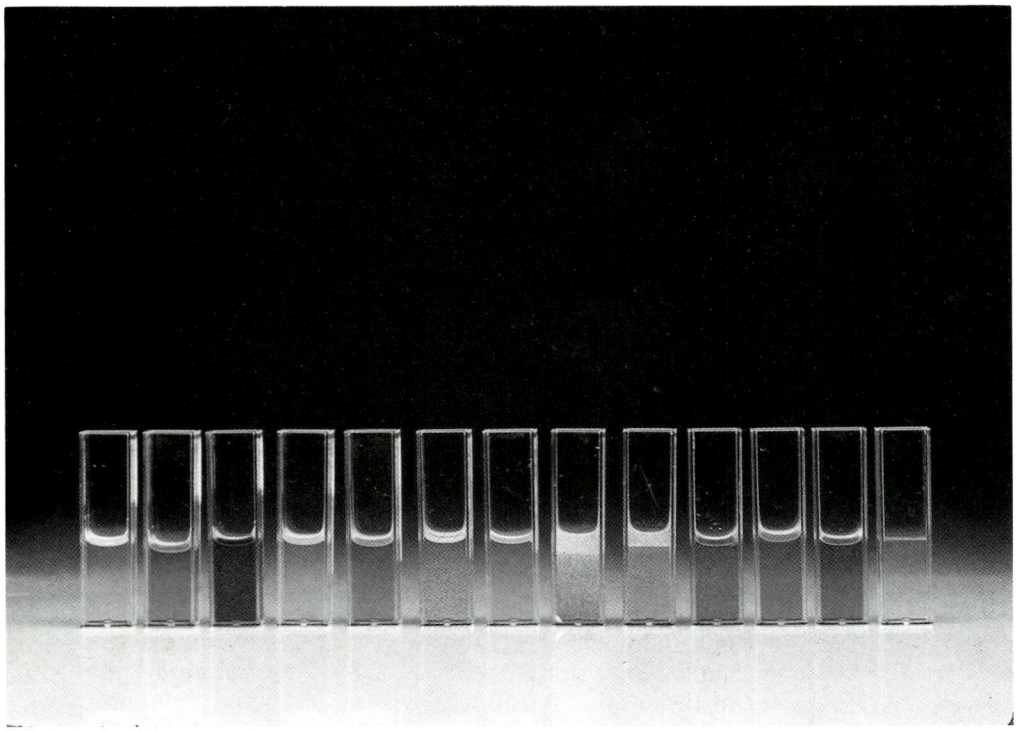

Fig. 1 Polystyrene cuvettes containing the mouthwashes, prior to mixing. From left to right: Listerine®, Eludril, Act®, Cepacol®, Listermint™, Scope®, Corsodyl™, Odex, Odex+, Colgate® Tartar Control Mouthwash, Veadent®, Signal®, water. (See colour plate).

cuvettes rinsed in water alone. Other commercial mouthrinses tested removed between 15% (Corsodyl ™) and 50% (Listerine®) of the bacterial film. Desorption by both oil: water formulations was significantly greater ($P < 0{\cdot}005$) than that observed for all the other mouthwashes.

DISCUSSION

The present report shows that oil:water formulations containing CPC or CPC and CHX, have the capacity to desorb large proportions of bound microorganisms from surfaces as compared to conventional mouthwashes. It has been previously shown (Rosenberg *et al.*, 1983) that aqueous:olive combinations removed over 97% of bacterial film from polystyrene cuvettes, compared to control cuvettes rinsed with water alone. In that study, desorption was measured by subsequent outgrowth of the remaining cell layer. However, since commercial mouthrinses routinely contain antibacterial agents, the outgrowth approach precluded comparison of the desorption efficacy of oil: water mixtures with commercial preparations. Furthermore, a much higher initial concentration of cells was used in the present study, resulting in a more recalcitrant biofilm. Finally, the presence of the cationic antibacterial surface active agents, cetylpyridinium chloride and chlorhexidine, in the oil:water formulations did not

Table 1 Mouthrinses Used in This Study

Name of Mouthwash	*Manufacturer*
Listerine®	Warner-Lambert Co., Morris Plains, NJ 67950
Eludril	PF Medicament, 125, rue de la Faisanderie, Paris, France
Act®	Johnson & Johnson, New Brunswick, NJ 08903
Cepacol®	Merrell Dow Pharmaceuticals Inc, Cincinnati, Ohio 45215
Listermint (trade mark)	Warner Lambert Health Care, Eastleigh, Hants, England
Scope® (Peppermint)	Proctor & Gamble, Cincinnati, Ohio 54202
Corsodyl (trademark)	Imperial Chemical Industries, PLC, Macclesfield, Cheshire, Great Britain
Odex	Agis Ltd, Tel-Aviv
Odex +	Agis Ltd, Tel-Aviv
Colgate® Tartar Control Mouthwash	Colgate Palmolive Co., NY, NY 10022
Veadent®	Glaxo (under license from Vipont Laboratories Inc), Fl. Collins, USA
Signal®	Lever Brothers Co., 390 Park Avenue, NY, NY 10022

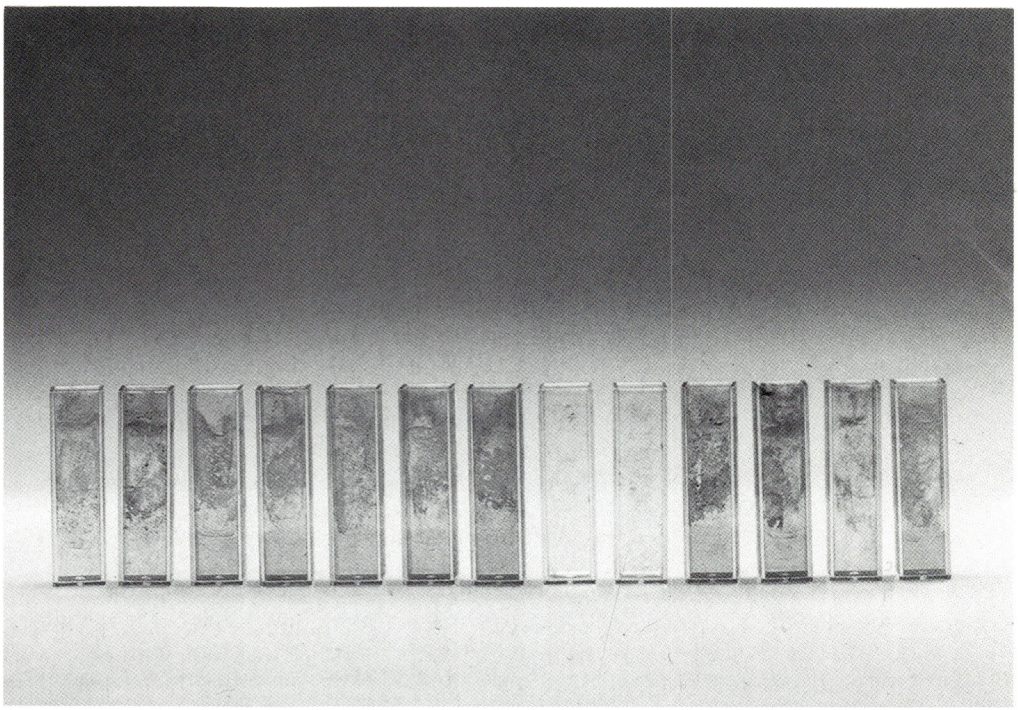

Fig. 2 Stained residual bacterial layer, following rinsing procedure. Cuvettes were layered with bacterial film, washed, rinsed with mouthwashes and stained as described in Materials and Methods. Order of cuvettes is the same as Figure 1. (See colour plate).

Table 2 Desorption of an Adherent Bacterial Film by Mouthwashes

Name of Mouthwash	*Percent Desorption compared to water control*[a]
Listerine®	50
Eludril	34
Act®	40
Cepacol®	40
Listermint (trademark)	36
Scope® (Peppermint)	28
Corsodyl (trademark)	15
Odex	98
Odex+	92
Colgate® Tartar Control Mouthwash	21
Veadent®	28
Signal	44

[a]Average of 2 readings

adversely affect the desorption properties. Addition of either of these agents to oil: water mixtures results in long-term reductions in oral malodour and microbial levels (Y Konis & M Rosenberg, unpublished). It should be emphasized that commercial mouthrinses containing cetylpyridinium chloride (e.g. Cepacol®, Scope®), or chlorhexidine (e.g. Eludril, Corsodyl (trademark)) were relatively ineffective in desorbing the bacterial layer. Indeed, Odex preparations are relatively ineffective desorption agents when the oil phase is excluded.

The present study is based on an *in vitro* technique based on desorption from a smooth, hydrophobic surface. Whereas hydrophobic interactions are important for oral adhesion, they probably act in concert with other mechanisms. Nevertheless, hydrophobic inhibitors are able to block adhesion to oral surfaces and to desorb adherent cells (Nesbitt *et al.*, 1982; Rosenberg and Kjelleberg, 1986; E Rosenberg *et al.*, 1983). Moreover, whereas the well-characterized model organism employed here (RAG-1) is not an oral microorganism, ODEX also effectively desorbs *Actinomyces viscosus* and *Streptococcus sanguis* from polystyrene tubes (not shown). Furthermore, microscopic observation of ODEX following intraoral use shows that high levels of oral microorganisms are concentrated on the surface of expectorated oil droplets (Rosenberg, 1990*b*).

Finally, *in vitro* experiments indicate that rinsing with ODEX removes more stainable oral debris than rinsing with the aqueous phase of ODEX alone. These findings suggest that ODEX also has *in vivo* oral desorption capabilities and that the oil phase is critical for the desorbing effect.

The above notwithstanding, it is likely that efficient bacterial desorption is only one of several criteria to be considered when discussing mouthwash efficacy. For example, whereas Corsodyl™, a chlorhexidine mouthrinse, is highly effective in reduction of microbial levels (Perdok *et al.*, 1989) and oral malodour (Rosenberg, 1990*a*), in the

present report, it exhibited the poorest desorption capacity among all mouthwashes tested. We can consider the following hypothetical example: a nonbacteriostatic, nonbactericidal mouthwash that desorbs 75% of oral microorganisms (a daunting challenge, considering the concentration of oral microorganisms in multilayered plaque and sequestered subgingival sites) can be effective for only two generations, since only two doublings are required to regain original microbial levels. However, a mouthwash that desorbs 75% of the oral microbiota and kills 75% of the residual microorganisms would be three times as effective. Thus, a formulation such as ODEX+, which is still an effective desorber (92%), yet contains a cocktail of antibacterials, including CHX, may have high efficacy. *In vivo* experiments to test the long-range effects of such oil:water formulations on oral microbial levels and general oral hygiene and health are thus warranted.

Acknowledgement

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Colour Plate 1 (See Goldberg & Rosenberg "Bacterial Desorption by Mouthwashes . . .")

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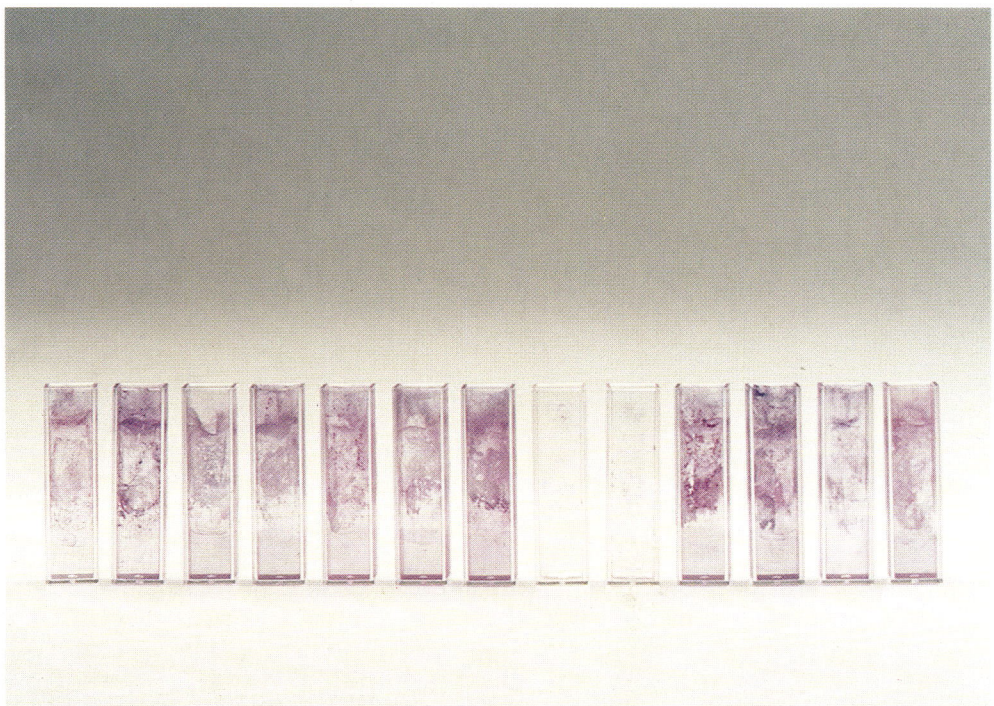

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